Research Protocol on Comparative Assessment of Tissue Regeneration by Ayurveda Medication (Amalaki Patra Mashi Ointment) and Silver Sulfadiazine in Dagdha Vrana (Burn Wound)

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Skin is the largest organ in the body and performs a wide variety of different function. It play an important role in the injury of the skin. Burns (Dagdha Vrana) are significant health challenge and healing can result in scar formation. Within the herbal medicine tradition the concept of Tonification and Trophorestoration is well established. Concepts of regeneration and Trophorestoration in Ayurveda provide another dimension to the area of regenerative medicine. Acharya Charaka suggests to use drugs of Amalaki to prevent Vrana. Amalaki and Silver Sulfadiazine (SSD) are the two comparative drugs chosen to evaluate the Trophorestoration concept through Dagdha Vrana in Albino rabbits.

Materials and Methods: After identification and phytochemical study of test drugs 18 adult Albino rabbits will be divided into 3 groups with six Albino Rabbits in each group. The burn wound will be....

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induced by using metal disc, three groups have been taken in which first is control group, second is standard control, third is experimental group. Before applying experimental drugs to respective group the tissue sample will be taken from each of albino rabbits and from one of the healthy tissue sample of normal skin will be taken by Punch Biopsy for histopathological assay. Examination of wound area, swelling, redness, oozing will be observed. Macroscopic and Microscopic assessment of wound will be done on day 0th, 4th, 8th, and 15th, 21st day after burn. Simultaneously on 0th day and 21st day SOD and MDA test will be done. The remaining animals will be returned to the animal house for their reuse.

**Expected Results:** Whether Amalaki Patra Mashi ointment has more effect on troporestoration property of Twacha in Dagdha Vrana (Burn wound) than Silver Sulfadiazine.

**Keywords:** Dagdha Vrana; Sapta twacha; Trophorestoration; Amalaki.

### 1. INTRODUCTION

Burns (Dagdha Vrana) are significant health challenge and healing can result in scar formation [1]. “Burn is defined as tissue damage caused by a variety of agents such as heat, chemicals, electricity, sunlight, or nuclear radiation. The most common are burns caused by scalds, building fires and flammable liquids and gases. Thermal burn and related injuries have remained a major cause of death and disability. Although small burns are not usually life threatening, they need the same attention as large burns, in order to achieve functional and cosmetic outcome” [2]. “Wound is defined as disruption of cellular, anatomical, and functional continuity of a living tissue. It may be produced by physical, chemical, thermal, microbial, or immunological insult to the tissue. When skin is torn, cut, or punctured it is termed as an open wound and when blunt force trauma causes a contusion, it is called closed wound, whereas the burn wounds are caused by fire, heat, radiation, chemicals, electricity, or sunlight” [3].

“Wound closure represents a primary goal in the treatment of very deep large wounds, for which mortality rate is particularly high. However, the spontaneous healing of skin eventually results in the formation of epithelized scar and scar contractures (repair) which might distort the tissues and cause lifelong deformities and disabilities. These clinical evidences suggest that wound closure attained by means of skin regeneration instead of repair, should be the true goal of burn wound management” [4].

“Mashi Kalpana is an important pharmaceutical preparation mentioned in Ayurveda Pharmaceuticals. Mashi Kalpana is the form of medicine which can be prepared by heating herbal or animal content upto transfer into carbonized form. It is used externally as well as internally. It is cost effective, less time consuming preparation and having quick result” [5]. Rasa - Tarangini, Rasashastra treatise 20th century enumerated various types of Malahar Kalpana based on Yogratnakar mentioned Malahar Kalapana, it removes Mala (residue) etc. from wounds etc. this is similar to ointments in modern pharmaceutics Malahar Kalpana. Malahar has a property like Snehan (Oleation), Cleansing, Ropan (Healing), Lekhan (Scraping) and Varnya (Beautifying) [6]. Its have its own therapeutic advantages like easy pharmaceutical procedure ease of mode of application and higher shelf-life period [7].

The various Dravyas are used for applications to cure Dagdha Vrana. Bhavaprakash and Acharya Charak suggested that Out of all Rasa Kashay Rasatmak Dravyas are useful for Vrana Ropan [8]. Amalaki (Emblica officinalis) (EO) is a rich source of vitamin C, which is a potent antioxidant [9]. “It is foremost amongst the anti-aging drug (Vayasthapan) or best amongst the rejuvenating herbs; it has properties like Rasayana (adaptogenic), ajara (usefulness in pre-mature aging), Ayushprada (prolongs cell life), Sandhana karaka (improves cell migration and cell binding), Kantikara (improves complexion)” [10]. According to Acharya Charaka, Kashay Rasa also having the properties of Sanshaman (palliative), Sangrahi, Sandhan (to hold), Pidan (Pain killer), Ropan (Healing property of Vrana), Shoshan (to absorb), Kledan (to provide moisture). It also pacifies the Pitta and Kapha Dosha [11]. “Many researches are found the extractions of the Amalaki leaves are used for wound healing purpose. Leaves contains gallic acid, chebulic acid, ellagic acid, chebulinic acid, chebulagic acid, amlic acid, alkaloids phyllantine and phyllantidine etc. These phyto-Chemicals having capacity of biological activities like antioxidant, antimicrobial, anti-inflammatory, antidiabetic, antitissuive, anti-radio protective, chemo preventive, wound healing activities and so on” [12]. “Traditional system of medicine like
Ayurveda which are known for their healing capabilities can offer a lot more to the science of regenerative medicine. Trophorestoration is the path to repair and regeneration. Within herbal medicine tradition notion of tonification and trophorestoration are well established" [13]. The present study will be carried out to explore the concept of trophorestoration of tissues by the test ointment with and without the treatment in second degree thermal burns and compare it with ointment Silver Sulfadiazine.

2. MATERIALS AND METHODS

2.1 Materials

This study will be conducted under following headings.

2.1.1 Experimental animal

All 18 albino rabbits weighing 3 to 3.5 kg will be used in this study. All albino rabbits will keep in a temperature-controlled (25 ± 1°C) environment with a 12-h light/dark cycle and kept in individual cages. They will feed with fresh hay, water, and fresh vegetables and given water ad libitum will be obtained from Central Animal House of DMIMS [14]. All experimental protocols involving the use of animals will be conducted in accordance with the CPCSEA guidelines after the approval of institutional animal ethical committee.

2.1.2 Selection of material/drug

The plant of Amalaki and other material will be identified authenticated and from Dravyaguna Department of Mahatma Gandhi Ayurved College Hospital and Research Centre, Wardha.

2.1.3 The material/Drug: (Both the experimental drugs will be freshly prepared)

- Amalaki Patra in the form of Mashi
- Coconut oil - 100% standard virgin pure oil
- Silver sulphadiazine1% 25 gm will be used which is procured from AVBRH pharmacy

2.2 Methods

Experimental Study

2.2.1 Preparation of animal models

The albino rabbits will be acclimatized to laboratory conditions for one week prior to the experiment. The albino rabbits will be anesthetized with single intramuscular injections, xylazine 2.5-10 mg/kg IM, ketamine 22-50 mg/kg IM.

2.2.2 Thermal injury

1. The area on the back of the rabbit was shaved and animal kept for fasting overnight. The next day the animal was anaesthetized using Ketamine in the dose of 50 mg/Kg of body weight I.M. (1 ml/kg of body weight). A metal disc of wt. 50 gm, diameter 2.5 cm (25mm), thickness 1.1cm (11mm) and area 4.910 sq. cm (491.07 sq. mm) was heated in the blue portion of the flame of spirit lamp for 5 minute and then immediately kept on the shaved part for 30 seconds with minimal pressure. This method was found to be more accurate and convenient in producing the second degree burns in comparison with the molten wax method. The scientific paper in this regard was presented in 56th annual national conference of physiologist and pharmacologist of India, APPICON 24th December 2010, JNMC, Sawangi, Maharashtra.

2. Administration and Application of Drugs: Standard Ointment Silver Sulfadiazine and New Herbal Ointment was applied daily on the burn wound.

3. Fluid resuscitation: All animals will be immediately resuscitated with lactated Ringer’s solution (2 ml/100 g body weight) applied intraperitoneally to prevent dehydration.

4. Cooling effect: After Burn the area of burn will immediately cooled by Running water and cold water. (2 to 15°C).

5. Local anesthesia: To prevent pain Novocain 5 % ointment will be used.

6. The burn should subsequently be covered with a sterile, occlusive, non-adherent dressing to reduce pain, limit contamination, and prevent further trauma.

2.2.3 Preparation of the material/drug

Preparation of Amalaki Patra Mashi will be done on the basis of classical methods given the text of Ayurveda.

- Amalaki Patra Mashi ointment
Preparation of the ointment will be done as per Malhar Kalpana.

2.2.4 Anatomical assessment criteria for burn wound

Microscopic assessment of Dagdha Vrana

1. According to ayurveda parameters and Grades
   - Varna (colour)
   - Strav (secretions)
   - Gandha (smell)
   - Akriti (Floor & Granulations)

2. Assessment according to Vrana on the day 0th, 4th, 8th, 15th and 21st

3. Wound contraction rate will be calculated according to formula-

\[
\text{Wound contraction rate} = \frac{\text{Original wound area} - \text{specific day wound area}}{\text{Original wound area} \times 100}.
\]

4. Criteria for grading of epithelial regeneration

Microscopic Assessment of Dagdha Vrana

1. Histopathology: Punch biopsy [15].
2. Parameters of histological assessment of wound.
4. Blood investigations - SOD and MDA levels.

2.3 Method of Data Collection

Data will be collected by laboratory reports and histology reports.

2.4 Statistical Analysis

The thickness of granulation tissue will be examined at the center of each wound and recorded. The data were expressed as mean ± standard errors (SEM). Differences between group means and between days four, nine, and 14 were estimated using a one-way analysis of variance (ANOVA) and a Duncan test was performed for multiple comparisons using the SPSS 12.0 for Windows. Results were considered as statistically significant at \( P < 0.001 \).

2.5 Expected Results

To find out better drug in Dagdha Vrana (Burn wound) through Trophorestoration concept of Ayurveda wrt Twacha Sharir.

3. DISCUSSION

The healthy regeneration of tissues is based on the fundamental concepts of Ayurveda which encourages tissue regeneration Santarpan (nourishing), Jivaniya (life promoting), Bruhan (Bulk promoting), Ropan (Healing), Sandhan (Unifying), Tarpan (Nutrient Provider), Preenan (Nutrient Provider) [15-17]. Amalaki acts as a both Vayasthapan and Rasayan Herb which having restoration property [18]. The concept of Trophorestoration which is the path to repair and regeneration is well established in Ayurveda through herbal medicine through herbal medicine [19]. Modern medicine is yet to explore conceptual aspects of the regenerative medicine. Hence it is need to explore Trophorestoration concept of Ayurveda based on fundamental modalities in case of Dagdha Vrana compare with modern medicine [20].

<table>
<thead>
<tr>
<th>Groups</th>
<th>Intervention</th>
<th>Drug</th>
<th>Quantity for local application</th>
<th>Sample Size</th>
<th>Duration</th>
<th>Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A Placebo</td>
<td>No intervention will be done</td>
<td>No drug</td>
<td>-</td>
<td>6</td>
<td>21 Days</td>
<td>LA</td>
</tr>
<tr>
<td>Group B Standard control</td>
<td>Standard Drug</td>
<td>Silver Sulfadiazine (SSD)</td>
<td>1 Angula i.e</td>
<td>6</td>
<td>21 Days</td>
<td>LA</td>
</tr>
<tr>
<td>Group C Experimental</td>
<td>Study Drug</td>
<td>APM (Amalaki Patra Mashi Ointment)</td>
<td>2 Cm thick (Sha. (Sa.UK.74-75)</td>
<td>6</td>
<td>21 Days</td>
<td>LA</td>
</tr>
</tbody>
</table>
4. CONCLUSION

Conclusion will be made on the basis of result of Statistical Analysis and histopathology reports.

CONSENT

It's not applicable.

ETHICAL APPROVAL

Protocol is approved by institutional animal ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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